Proliferation of an isolated wheat culture under the influence of hormonal factors against the background of salt stress

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The induction of callus formation in three wheat genotypes from immature embryos has been studied. The obtained callus strains were cultivated against the background of chloride salinity for two passages in the presence of various hormones of auxin nature. Different efficiency of 2,4 D, NAA and IAA on the formation of morphogenic zones against the background of a stress factor has been established. It had been found that 2.4 D was the most effective inducer of proliferation with subsequent induction of morphogenesis.

Keywords: *In vitro, phytohormones, exogenous auxins, proliferation, stress factor, hormonal background*

INTRODUCTION

Methods of plant tissue culture have become an integral part of progress in applied and fundamental research of plant biology, thanks to the possibility of a detailed study of their development.

As a result of the cultivation of individual cells or poorly differentiated organs of plants isolated from the mother's body, a new biological system is created. In this system, cells, as a separate organism, are capable of independent development.

In vitro plant systems are convenient models for investigating the complex mechanisms underlying proliferation, cell differentiation, histogenesis, organogenesis, embryoidogenesis, and regeneration of the whole organism from cultured cells with totipotency (Eds et al., 2008; Javed, 2002).

Advances in the cultivation of cells, tissues and organs in the *in vitro* system have led to the development of fundamentally new technologies aimed at creating improved genotypes of agricultural plants with a high potential for adaptation to environmental stress factors while maintaining and increasing their productivity (Nikitina et al., 2015; Hemaid et al., 2013).

The priority area of applied biotechnology for creating stress-resistant plants is the cultivation and selection *in vitro* of somatic cells in a selective nutrient medium. The choice of a selective system *in vitro* is based on the mechanisms of plant resistance to one or another stress exposure.

Selected agents added to the nutrient medium may cause induced mutations in a certain part of the cells, which determine resistance to specific stressors. In a culture of isolated cells, the range of variability is greatly enhanced, which expands the possibilities of selection.

Cell selection and the resulting variability during *in vitro* cell cultivation are based on the stress factor of culturing conditions, which influences the processes leading to epigenetic variability in cultured cells and, in rarer cases, to various mutations.

The “range” of all emerging variants of variability is determined by the characteristics of the genome, that is, the genotypic characteristics of plant cells exposed to stress, the ability of the genome to repair and recover after cessation of exposure, as well as the fixation of the changes that can be of both genetic and epigenetic nature in the offspring. Promising model systems in this area of research are callus cultures *in vitro.* Such important in agricultural terms as cereals and legumes.

Numerous experimental data indicate that the induction of callus formation is largely

determined by the physiological status of the explant at the time of inoculation on the nutrient environment, as well as cultivation conditions, the most important of which is the optimal concentration of phytohormones (Davies, 2010).

Sufficient experimental material has been accumulated to study the effect of phytohormones on the induction of callus formation in vitro culture of cereal explants, mainly embryos and anthers. It was established that during *in vitro* cultivation on an induction medium, there occurs the dedifferentiation of the initial specialized or meristematic explant cells with their conversion to callus. This process is associated with the structural reorganization of the original cells and the induction in them of the ability to consecutive divisions with the final proliferation of cells. In general, the question of callus cell reprogramming is being addressed in the context of the general problem of genome variability in the process of dedifferentiation and *in vitro* callus formation (Harrison, 2012; Strnad et al., 2012; Sugiyama, 2015).

Of particular importance are studies on the preparation of altered forms cultivated against a stressful background. In this case, in order to obtain proliferating cells retaining competence for morpholgenesis of cells, studies on the selection of culturing conditions, primarily hormonal factors, are acquired.

The aim of this research was to study the features of proliferation processes against the background of such a stress factor as NaCl, when modeling chloride salinity and the influence of hormonal factors and their concentrations on these processes.

MATERIALS AND METHODS

The objects of research were 1 variety of *Triticum Durum* Desf. and 2 varieties of *Triticum Aestivum* Lş for callus induction, immature embryos were used, which were passaged onto Murashige and Skoog medium (MS) (Murashiqe et al., 1962), containing 0.8% agar, 3% sucrose. The extraction time for the immature embryo was 13-17 days after pollination. By this time, the size of the embryo was 0.8-1.5 mm. Introduction to culture and passaging was carried out as described previously (Mamedova et al., 1993).

2,4-D (dichlorophenoxyacetic acid) - 2 mg/*l* was used as callus inducers. Cell cultures were grown in the dark at a temperature of 26±1°C, replanting every 24-30 days on fresh nutrient medium during 2 passages. Murashige-Skoog MS medium containing 0.7% agar, 30 g sucrose, was used as a selective system. supplemented with 0.6 and 0.8% NaCl concentrations to induce osmotic stress. The hormonal background was created using various hormonal factors of the auxin nature of NAA (naphthylacetic acid) -2 mg/*l*; IAA (indolylacetic acid) - 2 mg/*l*.

At the beginning and end of cultivation, in all variants, the weight of approximately the same mass of callus cells was fixed under aseptic conditions. The presented results are the average weight values before and after passaging from 3 replicates and were expressed as a percentage of the control variants of each variety.

RESULTS AND DISCUSSION

According to the available experimental data, callus in the studied varieties of *Triticum aestivum* and *Triticum durum* originates from epidermal cells of the scute of the immature embryo of the optimal development phase (organogenesis phase, periodization, which is characterized by a certain cytological and histological status and a certain content of endogenous auxin IAA. It is fundamentally important that in this phase the epidermal cells of the scute are not covered by a dense cell wall.

The concentration of exogenous auxin, which determines the content of endogenous auxin (IAA) in immature embryos plays the main role in the induction of callus formation, while the content of this phytohormone is different for each of the varieties. Apparently, the immature embryo of this particular phase of development has the necessary degree of cell differentiation, which is expressed in the competence of epidermal cells of the scute to external hormonal influences. It is likely that the gradients of all hormonal and trophic factors present in all tissues and organs of immature embryos influence the process of formation and growth of callus.

Table 1. The effect of various concentrations of NaCl on the intensity of callus proliferation in the presence of hormonal levels (2.4; NAA; IAA) (in percent of the control)

Varieties	Control 2,4D	NaCl											
		100 mM		150 mM		100 mM		150 mM		100 mM		150 mM	
		2,4D				NAA				IAA			
		passages											
		I	II	I	II	I	II	I	II	I	II	I	II
Barakatli 95	99	95	90	90	83	65	65	60	58	55	50	60	53
Nurlu-99	94	92	88	84	80	62	63	54	55	52	58	44	50
Qobustan	94	85	87	80	80	55	52	55	55	50	50	50	50

**Control – variant with 2,4D.*

The content of endogenous auxin (IAA) in the cell mass depends on many factors and is primarily controlled by the presence of auxin oxidase (IAA oxidase), the activity of which, in turn, is determined by a number of other factors. That is why the study of proliferation processes, accompanied by the most active formation and development of embryogenic sites in the cultivated mass of callus cells, against the background of other synthetic and natural exogenous factors of a hormonal nature in the presence of a stress salt factor is the subject of interest.

According to our observations, all tested varieties had a rather high ability to initiate callus cells. In all investigated genotypes, the simultaneous formation of callus from immature embryos placed *in vitro* was observed. Intensive dedifferentiation of explants and the onset of callus formation in circumstances without the stress factor were observed already on the 5-7th day.

On average, the frequency of callusogenesis ranged from 95% to 100% depending on the genotype. After passage 0, callus cells were transplanted onto the medium with a stress factor in the presence of 2,4 - D; NAA; IAA (table 1).As can be seen from the table, in the presence of a stress factor, the growth of callus cells slowed down in all genotypes. During the 1st passage, the best results for growth indicators belonged to variants with 2.4D, variants with NAA and IAA by approximately 30 and 40%, respectively. At this stage, this was possibly due to the fact that 2.4D cultivation from the very beginning of callus induction ensured the accumulation of this hormone in cells and did not require a significant restructuring of the mechanisms that regulate the growth and development of cell masses. However, subsequent passaging also showed that variants with 2.4 D were more effective than variants using NAA and IAA. During the 2nd passage, the influence of NAA and IAA slightly increased compared to the first passage and amounted to approximately 25 and 30% of the activity of 2,4D.

With an increase in the NaCl content to 150 mM, the suppression of proliferation increased. However, the percentage ratio between the first and second passages was approximately the same, which can be explained by the acquisition of adaptability to the presence of an increased NaCl content over the entire cultivation period.

Considering the effectiveness of the formation of morphogenic zones, the effect of 2.4 D against the background of stressor phosphor was also more effective than in other variants.

It is quite possible this may be due to the fact that the initial trigger role of 2,4D initially ensured the mechanism of proliferation and the induction of the formation of morphogenic zones in the callus mass of cells, determined the specialization of a number of cells with competence in the synthesis of endogenous IAA, providing a pool of endogenous IAA with greater efficiency, even taking into account the activity of IAA oxidases.

CONCLUSION

Thus, we found that the most effective inducer of proliferation is 2.4 D at all stages of the experiment. The concentration of 2.4D 2 mg/*l* ensured normal callus proliferation, followed by the induction of morphogenesis, which allows the use of this concentration for cell engineering in

the production of plants with altered traits during cell selection for chloride salinity.

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Duz stresi fonunda hormonal amillərin təsiri altında təcrid olunmuş buğda kulturasının proliferasiyası

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Üç buğda genotipində yetişməmiş rüşeymdən kallus əmələ gəlməsinin induksiyası öyrənilmişdir. Əldə olunan kallus ştamları xlorid duzluluğu fonunda auksin təbiətli müxtəlif hormonların iştirakı ilə iki subkultivasiya müddəti becərilmişdir. Stress faktoru fonunda morfogen zonaların meydana gəlməsinə dair 2,4 D, IAA və IAA-nın fərqli effektivliyi müəyyən edilmişdir. Kallusun proliferasiyası və sonrakı morfogenez induksiyası üçün ən effektiv induktorun 2.4 D olduğu müəyyən edilmişdir.

Açar sözlər*: *İn vitro, fitohormonlar*, *ekzogen auksinlər, proliferasiya, stress amili, hormonal fon